Pheromonal Response in Brook Trout

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PHEROMONAL RESPONSE IN BROOK TROUT

A Research Report Submitted to:

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INTRODUCTION

Fisheries managers sometimes need to either (1) remove undesirable fish species from a lake or stream to reduce competition with other species, or (2) collect reproductively mature fish from a wild population. For example, non-native brook trout (*Salvelinus fontinalis*) compete with native cutthroat trout (*Oncorhynchus clarki*) in high-elevation streams in the Medicine Bow National Forest of southeastern Wyoming. Removal of the brook trout might help stabilize cutthroat trout populations or expand their current range. Moreover, fisheries managers would like to be able to capture and spawn reproductively mature trout from streams in the Medicine Bow National Forest as a source of fertilized eggs for hatchery rearing.

Current methods to remove undesirable fish include using toxicants or electrofishing, and the main method for capturing fish for spawning is electrofishing. However, the use of toxicants is not favored from an ecological perspective and has caused increased public concern in recent years. Although electrofishing is more acceptable from public and ecological perspectives, it is labor-intensive and usually not efficient. Thus, development of a nonlethal, species-specific, and more efficient capture technique for fish would be desirable.

Reproductively mature fish usually are attracted to other reproductively mature fish of the same species. For example, Sveinsson and Hara (1995) demonstrated that mature male Arctic charr (*Salvelinus alpinus* -- in the same genus as brook trout) release F-type prostaglandins that attract mature females and stimulate their spawning behavior. Moreover, a commercially available prostaglandin ($PGF_{2\alpha}$) attracted reproductively mature adults in the same study. Thus, Dr. Mike Young (USDA Forest Service, Rocky Mountain Research Station, Laramie, Wyoming) hypothesized that brook trout might also be attracted to $PGF_{2\alpha}$, and that this pheromone could be used to attract fish to nets in streams. For comparison to results of field studies being conducted by Dr. Young, we tested the ability of $PGF_{2\alpha}$ to attract brook trout in a controlled laboratory setting.

MATERIALS AND METHODS

Organisms

Brook trout were electrofished from Telephone Creek (upstream from Highway 130) in the Snowy Range of the Medicine Bow National Forest on 22 June and 3 October 2000. In June, all fish captured were collected; whereas in October, only mature males and females (based on size and skin coloration) were collected. Traditionally, the last few weeks of September and the first few weeks of October are the height of the spawning season for brook trout in the Snowy Range.

During electrofishing on each collection day, the fish were placed in aerated stream water in a holding tank on the back of a pickup truck parked near the collection site. Later the same day, the fish were transported to the University of Wyoming's Red Buttes Environmental Biology Laboratory and transferred into hard well water at 7°C after being gradually acclimated to that temperature. The fish were later tested for attraction to $PGF_{2\alpha}$ or brook trout males in the same water (pH - 8.2, alkalinity - 220 mg/L as $CaCO_3$, hardness - 220 mg/L as $CaCO_3$, and total dissolved solids - 270 mg/L).

The brook trout collected in June were fed trout chow as soon as they were brought into the laboratory. Several days after being collected, they were weighed and measured and divided into two groups (small and large fish). On 6 July, we immersed the fish in Chloramine T for 30 minutes as a prophylactic disinfection. Eighty-nine small fish (<30 g) were held without further processing until the pheromone-attraction study began in October. In August, 120 large fish (>30 g) were injected with a hormone to accelerate their reproductive maturity (see next subsection). We fed the fish at 6% of their body weight per day until 25 August, when we decreased that ration to 3% because the fish were not eating the full 6% ration.

The mature male and female brook trout collected in October were sorted by gender and placed in separate tanks when they were brought to the laboratory. We gave them a 5-day acclimation period before the pheromone-attraction study began, and we fed them trout chow

while in captivity. These fish showed no signs of disease and were not given disinfected.

Hormone Injection

On 3 August, we injected Ovaplant into 120 of the large brook trout (30-100 g) that were collected in June. Ovaplant is a pelletized implant of salmon gonadotropin releasing hormone analog (sGnRHa; purchased from Syndel International Incorporated, Vancouver, British Columbia, Canada). After they received the implant, the fish were placed back into their holding tanks. The purpose of the sGnRHa was to hasten (1) recrudescence of the fishes' gonads and (2) their production of and receptivity to conspecific pheromones. The implants were injected intraperitoneally -- laterally and just anterior to the fish's vent using a RalGun Pellet Injector (Syndel International Incorporated). Before implanting the pellets, we cut the original 75-μg pellets approximately in half to reduce the dose to our relatively small brook trout. Use of Ovaplant was authorized under Syndel International's Clinical Field Study Protocol for Investigative New Animal Drug (INAD) Exemption (INAD Number 10-087, Study ID Number L0049006).

In mid-September, some of the sGnRHa-implanted fished developed fungal infections on their gills and began dying (see Results and Discussion). On 29 September, we treated the sGnRHa-implanted fish with formalin. However, sGnRHa-implanted fish continued to die. Because of this and an apparent lack of gonadal maturation, we did not use any of the sGnRHa-implanted fish in the behavioral attraction tests. Instead, we euthanized 10 non-injected fish and the remaining 16 sGnRHa-implanted fish on 27 September and inspected their abdominal cavities for (1) presence of an injection scar and an implant, (2) signs of infection at the site of the implant, and (3) degree of gonadal maturation. Degree of gonadal maturation was ranked according to the following classification system:

Females -- 1. Immature - ovaries transparent, small, and pink.
2. Resting - ovaries contain previtellogenic eggs only; transparent to pink.
3. Vitellogenic - opaque yolk-filled eggs clearly visible; ovaries orange-yellow, usually plump.
4. Hydration - clear, hydrated eggs visible among vitellogenic eggs.
5. Ripe - hydrated eggs present in large numbers in the center of the ovaries and can be squeezed out of the oviduct.
6. Spent - oocytes being resorbed; ovaries flaccid, bloody, and red; eggs may be present.

Males -- 1. Immature - testes small and white, perhaps distinguishable from ovaries by angular appearance.
2. Resting - spermatogenesis occurring; testes ivory white.
3. Mature - spermiation; viscous milt in duct.
4. Ripe - hydration; runny milt in duct.
5. Spent - testes grey, bloody, and flaccid.

But based on our visual examinations, we could not determine the gender of fish in Stage 1; therefore, we classified the gender of all immature fish as "unknown".

Attraction Study

We tested the attraction of three groups of brook trout (mature males, mature females, and juveniles) to $PGF_{2\alpha}$ and to mature male brook trout. Mature males were used as the attractant from 9 to 10 October, whereas $PGF_{2\alpha}$ was used as the attractant from 11 to 13 October. All tests were conducted at 7°C.

$PGF_{2\alpha}$ was tested as its tromethamine salt ($C_{20}H_{33}O_5 \cdot C_4H_{12}NO_3$, FW = 475.6, catalog #16020, Cayman Chemical, Ann Arbor, Michigan; provided to us by Dr. Mike Young). Because the $PGF_{2\alpha}$ came in crystalline form in glass vials (nominal 10 mg per vial), we added 1 ml of 95% ethanol to a vial to dissolve the crystals and produce a nominal 2×10^{-2} M solution of $PGF_{2\alpha}$.

We then diluted 0.1 ml of that ethanol solution 1:200,000 with Hydropure water to produce a nominal 10^{-7} M aqueous stock solution of $PGF_{2\alpha}$. That stock solution was pumped into the attractant end of the test chambers for the trials in which $PGF_{2\alpha}$ was used as the attractant. Because we mixed the $PGF_{2\alpha}$ stock solution (pumped at a flow rate of 1 ml/min using an FMI Model QG20 lab pump) with control well water (pumped at a flow rate of 1 liter/min) in each exposure chamber (see below), the nominal $PGF_{2\alpha}$ exposure concentration in the test chambers was 10^{-10} M; however, that concentration was not confirmed analytically.

The mature male brook trout used as attractants were randomly selected from the fish collected in the Snowy Range in October. We used mature males as an attractant to determine if the brook trout held in the laboratory would be attracted to conspecific pheromones. Five mature males were placed in a 60-liter fiberglass head tank through which well water flowed continuously. That attractant water (presumably containing pheromones exuded by the males) then flowed to the exposure chambers (see below). The males were acclimated to the head tank for $\geq$4 hours before testing began each day.

Two additional 60-liter fiberglass head tanks were used to deliver well water to the control ends of the exposure chambers. All three head tanks were plumbed to the exposure chambers so that an attractant could be introduced to either end of the chambers. Water depth in the head tanks (and, thus, head pressure in the tanks and flow rate of water out of the tanks) was held constant by float valves that controlled the flow of well water into the head tanks. Control and attractant waters flowed to their respective ends of the exposure chambers at 1 liter/min. The ends of the chambers to which the attractant water was delivered were randomly selected for each trial.

We conducted the behavioral-attraction tests in countercurrent-flow chambers similar to those used by Hansen (1998) in toxicant-avoidance studies with rainbow trout and chinook salmon. Briefly, the chambers were 15-cm-wide x 100-cm-long PVC troughs divided into control and treatment ends. Water (control or attractant) flowed into each end of the chamber and met at the center of the trough, where the water flowed out through nine 1.2-cm drain holes in the bottom of the trough. Dye experiments demonstrated that a steep vertical gradient between the waters flowing from each end of the chamber was established over the drain holes. Thus, each fish had a clear choice between control or attractant water.

Plastic screening was attached inside the troughs 12.5 cm from each end, and a 10-ml glass beaker was secured inside that space. We introduced control or attractant water into each end of the chamber by running it into the beaker, thus eliminating bubbles that might agitate the fish in the exposure portions of the chambers. Additionally, in the test in which $PGF_{2\alpha}$ was the attractant, the stock solution of the hormone was pumped into this beaker, ensuring adequate mixing with the well water before it entered the exposure chamber.

Three exposure chambers were arranged parallel to each other, with ~6 cm separating the troughs. The three exposure chambers and the three head tanks were surrounded by a black plastic curtain to decrease external disturbances, and a Panasonic Model WV-1410 CCTV video camera was mounted 3 m above the chambers to record fish movements during exposure to the attractants. A clock was placed next to the chambers and inside the field of view of the video camera to time the trials. Additionally, signs indicating the date of the test and the trial number were placed within the field of view of the camera. To avoid disturbing the fish, observers watched the trials on a video monitor outside the plastic curtain.

A behavioral trial consisted of a set of three fish tested simultaneously, one per exposure chamber. Although we attempted to test one mature male, one mature female, and one juvenile in each trial, misidentifications of gender based on external coloration sometimes altered that ratio. The intended gender of fish tested in each chamber was randomly assigned before each behavioral trial.

Each attraction trial consisted of four periods (the following description is modified from Hansen 1998:23): (1) a 20-min rinse period before and between tests with control water entering both ends of each chamber; (2) a 20-min habituation period commencing when one fish was placed into each chamber, (3) a 10-min latency period when the attractant-containing water was introduced into a randomly selected end of the chamber and allowed to establish a stable, steep

gradient between the control and attractant-containing water (with the fish already present in the chamber); and (4) a 20-min observation period when the behavioral data were collected. We did not consider a trial to be sufficient for this study if a fish did not move between the two ends of the chamber during the 10-min latency period and it made ≤3 roundtrips between the two ends of the chamber during the 20-min observation period.

Sample size originally was intended to be 10 fish per treatment, resulting in a total of 60 fish to be tested in the two-factor design (Factor 1: the attractant (male brook trout or $PGF_{2\alpha}$); and Factor 2: gender of the attracted fish (mature males, mature females, or juveniles)). However, we actually tested 11-12 fish in each gender category for their attraction to male brook trout; and based on the availability of more fish than anticipated after the attraction trials with male brook trout were completed, we tested 16-18 fish in each gender category for their attraction to $PGF_{2\alpha}$. Our inability to correctly distinguish among mature males, mature females, and juveniles based on external coloration led to unequal sample sizes. Additionally, the lack of sufficient movement between the control and treatment ends of the chambers by some fish forced us to exclude data from those fish, contributing even more to the unevenness of the sample sizes. In total, 82 fish were tested, of which the behavioral data from 11 were not analyzed (based on insufficient movement between the two ends of the chamber).

At the end of a behavioral trial, we anesthetized each fish, weighed and measured it, and gently squeezed its abdominal cavity to check for milt (males) or eggs (females) released from its vent. Fish that didn't release milt or eggs were classified as juveniles.

Data Analysis

After completing the tests, we reviewed the videotapes and continuously recorded the positions of the fish (i.e., in the control or the attractant side of the test chamber) during the observation period of each trial. The observations were automatically recorded, and the number of roundtrips between ends of the chamber and the percentage of time spent on each side of the chamber were calculated by the computer program AVOID5 that was written by Dr. James Hansen for analysis of toxicant-avoidance data (Hansen 1998).

We analyzed the data in three ways. First, we tested for normality of the data and homogeneity of the variances using the statistical package TOXSTAT Version 3.4 (WEST, Inc. and Gulley 1994). Second, because the data for percentage of time spent in the attractant water did not violate the assumptions of normality (chi-square goodness of fit test; $P < 0.01$) and homogeneity of variances (Hartley's test, Bartlett's test, and Levene's test; $P < 0.01$), we then used one-sample t tests (SPSS Version 8.0) to test for significant differences of the average percentage of time spent in the attractant water from 50% -- the percentage of time expected if the fish were neither attracted to nor avoided the attractant water. Finally, we used a general linear model (GLM in SPSS Version 8.0) to analyze the percentage of time spent in the attractant water (dependent variable) as a function of two random factors (attractant water and gender).

RESULTS AND DISCUSSION

Hormone-implanted Brook Trout

Lengths and weights of the brook trout collected from the Snowy Range on 22 June are presented in Appendix Table A-1, and a frequency distribution of their weights is shown in Figure 1. One hundred twenty of those fish were injected with an sGnRHa implant on 3 October.

One of the hormome-implanted fish died on 17 August and one died on 18 August, both of undetermined causes. On 5 September, the remaining hormome-implanted fish outwardly appeared to be healthy. Subsequently, eight of those fish died on 15 September, three died on 18 September, and eight died on 19 September, all without apparent infections. But on 26 September, 27 of the hormone-implanted fish were found dead with a fungal infection of the gills; and another 18 were found dead on 27 September, and 8 more were found dead on 28 September. Despite subsequent formalin treatment for the tentatively identified *Ichthyobodo*

infection, more hormone-implanted fish died until 27 October, when we euthanized and examined the remaining 16 fish. None of the non-implanted fish died during the same period in which the hormone-implanted fish died.

Reproductive status of sGnRHa-implanted and non-implanted brook trout is presented in Table 1. Of the eight hormone-implanted fish examined on 28 September, only one was reproductively mature (a female with 3-4 mm oocytes). Of the 16 hormone-implanted fish examined on 27 October, 50% were vitellogenic females with 1-2 mm oocytes; and the gender of the other 50% (whose reproductive organs were not developed) could not be determined. Because 50% of the 10 non-implanted fish also were vitellogenic females with 1-2 mm oocytes, the sGnRHa implant did not appear to accelerate the reproductive maturity in these brook trout. Instead, the implants (or the process of injecting the implants) appeared to increase the susceptibility of the fish to disease. More than 50% of the hormone-implanted fish examined on 27 October appeared to be starving, but none of the non-implanted fish appeared to be starving. This apparent starvation might also have increased the susceptibility of the hormone-implanted fish to disease.

Behavioral Attraction Tests

Raw data for brook trout exposed to male brook trout and $PGF_{2\alpha}$ as the attractant are presented in Appendix Tables A-2 and A-3, respectively. The standard lengths of the mature male and mature female brook trout used in the behavioral-attraction tests ranged from 112 to 183 mm (means = 150-157 mm), and their weights ranged from 20 to 89 g (means = 47-58 g) (Table 2). Although the 95% confidence interval for the weight of females exposed to $PGF_{2\alpha}$ did not overlap with the 95% confidence interval for the weight of females exposed to male brook trout, all of the other 95% confidence intervals for weight and all of the 95% confidence intervals for length overlapped. The juveniles used in these behavioral-attraction tests tended to be smaller than the mature males and females (lengths ranged from 112 to 173 mm, with means 138-142 of mm; and weights ranged from 20 to 97 g, with means of 41-42 g); however, the only non-overlap of 95% confidence intervals for juveniles was a difference in lengths between the juveniles and the mature males exposed to male brook trout.

Mature males, mature females, and juveniles tended to be slightly attracted to water in which mature male brook trout were held, but the percentage of time spent in the attractant water did not differ significantly ($P>0.05$) from 50% -- the percentage of time expected if the fish were neither attracted to nor avoided the attractant water (Table 3 and Figure 2). On the other hand, mature males, mature females, and juveniles tended to slightly avoid water containing 10^{-10} M $PGF_{2\alpha}$ (nominal concentration), but the percentage of time the mature males and the juveniles spent in the attractant water did not differ significantly ($P>0.05$) from 50% (Table 3 and Figure 2). Only the percentage of time spent in the $PGF_{2\alpha}$ water by the mature females was significantly less than 50% ($P<0.05$). A two-random-factor GLM model revealed that on average, (1) all three reproductive status/gender categories combined spent a significantly greater percentage of time in the water in which male brook trout were held than they did in the $PGF_{2\alpha}$ water, and (2) the amount of attraction or avoidance did not differ among the three reproductive status/gender categories (Table 4). Additionally, the interaction term between the attractant and the reproductive status/gender categories did not differ significantly (Table 4). The behavioral-attraction data for 1 male and 3 juveniles in the study of attraction to male brook trout and for 3 males, 2 females and 2 juveniles in the study of attraction to $PGF_{2\alpha}$ were excluded from the analyses because of insufficient movement during the acclimation and tests periods. These are the fish shown in Appendix Tables A-2 and A-3 that made 0 trips during the acclimation period.

Although the slight (but not significant) attraction for water in which males were held and the slight (but mostly not significant) avoidance of $PGF_{2\alpha}$ water is consistent with results of field studies in which mature male brook trout and $PGF_{2\alpha}$ have been used as attractants for brook trout in streams (personal communication, Dr. Mike Young, USDA Forest Service Rocky Mountain Research Station, Laramie, Wyoming), our laboratory results should be interpreted with caution. The mature males and mature females probably were still stressed from their capture in Telephone Creek and subsequent transport to Red Buttes Laboratory only five days prior to the

beginning of the behavioral-attraction tests. We chose a short holding time in order to attempt to retain the fish at the peak of their reproductive maturity and to avoid diseases that might infect the fish during a longer holding period. In future studies, earlier capture of the fish with a considerably longer holding time in the laboratory might help them acclimate to the laboratory. However, manipulation of the laboratory environment (e.g., appropriate changes in water temperature and light:dark cycles) might be needed to help the fish reach reproductive maturity and receptivity to conspecific hormones in the laboratory. Based on the injection technique and dose we used, the commercially available sGnRHa implant was not useful for hastening recrudescence in these relatively small fish.

At first, the result that the juvenile fish responded to the attractants almost identical to the mature males and females might appear to be inconsistent with expectations. But half of a small sample of that batch of juvenile fish (the 10 non-implanted brook trout in Table 1) were females in an intermediate stage of ovarian development (1-2 mm oocytes). Thus, a large percentage of the "juvenile" fish used in the behavioral-attraction tests probably were reproductively advanced and might have been receptive to conspecific hormones -- despite the fact that we could not squeeze eggs or milt from them after they were tested. In future studies, the reproductive status of juveniles must be tested more directly than just by checking for no release of eggs or milt when the abdominal cavity is squeezed.

Finally, we recommend that (1) the presence of reproductive hormones (and/or their metabolites) be quantified in attractant water flowing out of tanks in which mature fish are held, and (2) the concentration of $PGF_{2\alpha}$ (and/or other commercial hormones) in chemically prepared attractant waters be quantified in future studies. Without such quantification, the researcher will not know if the purported attractants were present in the exposure chambers at biologically relevant concentrations (or even present at all). Moreover, we recommend that behavioral attraction to ethanol (the carrier solvent in which the $PGF_{2\alpha}$ was initially dissolved) be tested, to determine if the tendency of brook trout to avoid $PGF_{2\alpha}$ was actually a tendency to avoid ethanol.

ACKNOWLEDGEMENTS

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Table 1. Reproductive status of brook trout (*Salvelinus fontinalis*) that were injected with an sGnRHa implant. The fish received the implant on 3 August 2000. The reproductive status of 8 sGnRHA-implanted fish was determined after they died of a fungal infection on 28 September; and the reproductive status of the remaining 16 sGnRHA-implanted fish was determined after we euthanized them on 27 October. For comparison, the reproductive status of 10 non-implanted brook trout was determined after they were euthanized on 27 October.

Date analyzed	Length (mm)	Weight (g)	Gender	Implant present?	Gonad stage	Comments
sGnRHa-implanted brook trout:						
28 Sept.	---[a]	---	Male	Yes	1	Died of infection; OK internally
28 Sept.	---	---	Male	Yes	1	Died of infection; OK internally
28 Sept.	---	---	Female	Yes	5	Died of infection; OK internally; 3-4 mm oocytes
28 Sept.	---	---	Female	Yes	1	Died of infection; OK internally
28 Sept.	---	---	Female	Yes	1	Died of infection; OK internally
28 Sept.	---	---	Male	No	1	Died of infection; OK internally
28 Sept.	---	---	Female	No	1	Died of infection; OK internally
28 Sept.	---	---	Female	No	1	Died of infection; OK internally
27 Oct.	168	58	Female	Yes	Early 3	OK internally; 1-2 mm oocytes
27 Oct.	132	23	Female	Yes	Early 3	Starving; OK internally; 1-2 mm oocytes
27 Oct.	142	37	Female	Yes	Early 3	OK internally; 1-2 mm oocytes
27 Oct.	140	33	Female	Yes	Early 3	Starving; OK internally; 1-2 mm oocytes
27 Oct.	132	25	Female	Yes	Early 3	Starving; OK internally; 1-2 mm oocytes
27 Oct.	153	37	Female	Yes	Early 3	OK internally; 1-2 mm oocytes
27 Oct.	142	32	Female	Yes	Early 3	OK internally; 1-2 mm oocytes
27 Oct.	164	42	Female	No	Early 3	Starving; OK internally; 1-2 mm oocytes
27 Oct.	162	30	Unknown	Yes	1	Starving; OK internally
27 Oct.	146	31	Unknown	Yes	1	Starving; OK internally
27 Oct.	142	29	Unknown	Yes	1	Starving; OK internally
27 Oct.	136	23	Unknown	Yes	1	Starving; OK internally
27 Oct.	130	22	Unknown	No	1	OK internally
27 Oct.	187	69	Unknown	No	1	OK internally
27 Oct.	193	92	Unknown	No	1	OK internally
27 Oct.	135	31	Unknown	No	1	Starving; OK internally
Non-implanted brook trout:						
27 Oct.	141	42	Female	No	Early 3	OK internally and externally; 1-2 mm oocytes
27 Oct.	121	27	Female	No	Early 3	OK internally and externally; 1-2 mm oocytes
27 Oct.	124	33	Female	No	Early 3	OK internally and externally; 1-2 mm oocytes
27 Oct.	138	37	Female	No	Early 3	OK internally and externally; 1-2 mm oocytes
27 Oct.	127	36	Female	No	Early 3	OK internally and externally; 1-2 mm oocytes
27 Oct.	152	55	Unknown	No	1	OK internally and externally
27 Oct.	128	33	Unknown	No	1	OK internally and externally
27 Oct.	140	41	Unknown	No	1	OK internally and externally
27 Oct.	124	31	Unknown	No	1	OK internally and externally
27 Oct.	130	32	Unknown	No	1	OK internally and externally

[a] --- = not measured.

Table 2. Summary of lengths and weights of brook trout (*Salvelinus fontinalis*) used in the behavioral-attraction tests.

		Length (mm)						Weight (g)					
Attractant	Gender	Mean	s.d.	n	Min.	Max.	95% C.I.	Mean	s.d.	n	Min.	Max.	95% C.I.
Males	Male	157	14.9	10	129	182	146.3 - 167.7	53	18.6	10	28	89	39.4 - 66.0
	Female	157	10.3	11	138	174	150.3 - 164.2	58	9.2	11	43	71	52.3 - 64.6
	Juvenile	138	10.5	11	121	160	130.6 - 144.7	41	19.5	11	25	97	28.1 - 54.3
$PGF_{2\alpha}$	Male	151	19.8	16	112	183	140.3 - 161.4	48	16.6	16	20	71	39.2 - 56.9
	Female	150	10.6	18	121	164	144.2 - 154.8	47	9.9	18	31	64	42.2 - 52.1
	Juvenile	142	16.9	16	112	173	133.5 - 151.5	42	19.1	16	20	89	32.0 - 52.3

Table 3. Percentages of time that male, female, and juvenile brook trout (*Salvelinus fontinalis*) spent in the attractant water when either male brook trout or $PGF_{2\alpha}$ was the attractant. These data are plotted in Figure 2.

Attractant	Gender	Percentage of time spent in attractant water	s.d.	n	95% C.I.	Range of values	Significance level[a]
Males	Male	60.6	14.8	9	49.1 - 72.0	34 - 78	0.066
	Female	56.0	24.9	11	39.3 - 72.7	14 - 87	0.442
	Juvenile	64.6	21.2	8	46.9 - 82.4	36 - 92	0.093
$PGF_{2\alpha}$	Male	51.6	20.0	13	39.5 - 63.7	17 - 83	0.776
	Female	37.0	17.0	16	27.9 - 46.1	11 - 64	0.008
	Juvenile	44.4	21.1	14	32.2 - 56.6	15 - 72	0.342

[a] Significance level (P value) for comparison of the mean with 50% -- the expectation if the fish were neither attracted to nor avoided the attractant water.

Table 4. Significance levels (P values) for the predictor variables in a general linear model in which percentage of time spent in the attractant water was the dependent variable, and attractant and gender were the predictor variables.

Variable	Significance level
Intercept[a]	0.819
Attractant	0.043
Gender	0.245
Attractant × Gender	0.600

[a] Significance of the intercept was calculated for a comparison with 50% -- the expectation if the fish were neither attracted to nor avoided the attractant water.

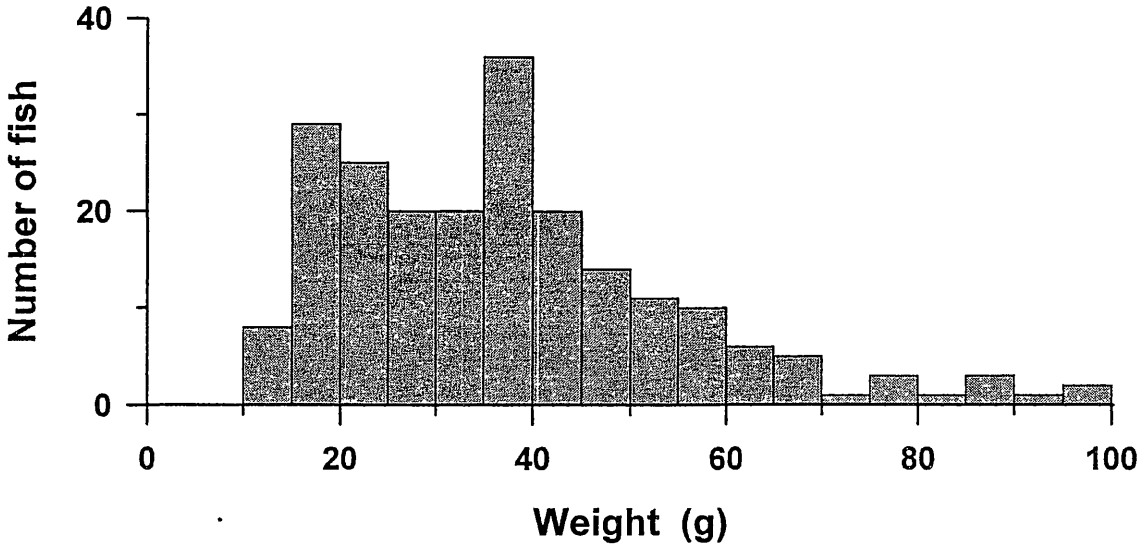

Figure 1. Distribution of the weights of brook trout (*Salvelinus fontinalis*) collected from Telephone Creek in the Snowy Range of the Medicine Bow National Forest in southeastern Wyoming on 22 June 2000.

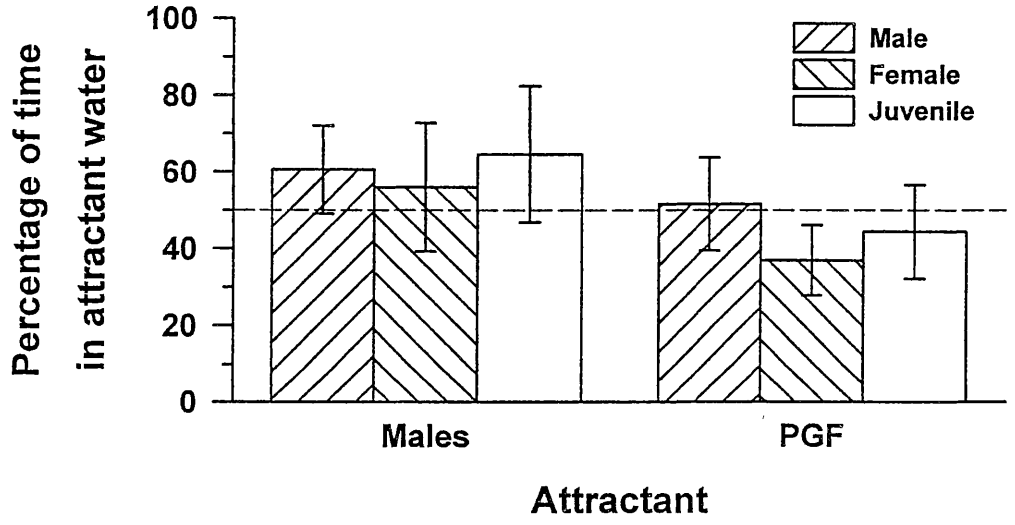

Figure 2. Percentages of time that male, female, and juvenile brook trout spent in the attractant water when either male brook trout or $PGF_{2\alpha}$ was the attractant. Error bars are 95% confidence intervals. The horizontal dashed line indicates 50% of the time spent in the attractant water -- the expectation if the fish were neither attracted to nor avoided the attractant water. These values are tabulated in Table 3.

APPENDIX TABLES

Appendix Table A-1. Lengths and weights of brook trout (*Salvelinus fontinalis*) collected from Telephone Creek in the Snowy Range of the Medicine Bow National Forest in southeastern Wyoming on 22 June 2000.

Fish #	Length (cm)	Weight (g)	Fish #	Length (cm)	Weight (g)	Fish #	Length (cm)	Weight (g)	Fish #	Length (cm)	Weight (g)
1	17.0	50.9	55	14.8	30.8	109	14.0	31.5	163	11.6	16.2
2	22.6	84.1	56	15.5	24.2	110	16.9	39.8	164	18.2	53.0
3	15.5	39.8	57	15.7	38.7	111	15.0	31.0	165	16.5	45.8
4	15.5	42.0	58	13.0	23.5	112	19.2	57.2	166	19.5	85.3
5	16.0	36.9	59	16.0	38.8	113	13.9	26.0	167	14.5	30.7
6	18.7	62.4	60	16.4	38.7	114	19.5	48.0	168	13.2	20.6
7	16.9	42.4	61	14.4	29.1	115	16.5	42.7	169	16.5	45.0
8	12.5	22.7	62	17.4	45.3	116	12.0	17.0	170	15.5	35.2
9	13.0	24.3	63	15.4	27.3	117	15.5	31.8	171	22.6	79.0
10	12.3	18.2	64	14.9	27.4	118	16.5	57.4	172	13.5	24.5
11	17.5	58.3	65	16.5	42.2	119	17.0	45.8	173	16.5	36.5
12	16.5	41.9	66	12.8	21.4	120	15.5	44.7	174	15.7	37.7
13	11.7	17.5	67	14.0	28.6	121	17.5	43.7	175	12.0	17.8
14	16.2	39.7	68	15.7	38.7	122	15.5	32.6	176	12.7	18.6
15	16.5	45.8	69	12.7	18.0	123	16.0	38.0	177	16.2	38.3
16	15.5	36.9	70	16.0	38.6	124	19.0	57.4	178	11.8	15.7
17	16.0	45.8	71	16.0	33.9	125	17.5	43.8	179	11.5	17.3
18	16.0	36.1	72	11.5	14.3	126	12.0	14.9	180	18.2	56.1
19	12.0	19.1	73	11.0	15.5	127	14.1	24.2	181	18.5	63.5
20	16.1	39.5	74	16.5	39.1	128	12.0	17.2	182	15.0	28.2
21	17.7	49.7	75	12.4	17.1	129	19.5	68.7	183	13.8	24.8
22	17.5	48.9	76	19.7	65.8	130	11.7	15.5	184	15.0	28.9
23	12.6	20.0	77	12.0	17.1	131	12.4	19.3	185	16.8	39.5
24	17.0	43.6	78	17.0	45.0	132	18.2	52.8	186	16.0	42.2
25	15.5	35.2	79	20.0	65.8	133	15.3	31.8	187	11.9	14.2
26	14.8	33.6	80	17.5	45.2	134	17.5	50.8	188	17.2	49.4
27	16.3	44.5	81	13.6	18.5	135	19.2	79.8	189	16.1	36.1
28	16.4	33.2	82	17.5	49.5	136	16.0	38.1	190	19.5	58.3
29	18.5	54.2	83	18.5	54.9	137	12.9	20.6	191	14.5	28.7
30	16.5	42.9	84	16.0	40.7	138	15.4	36.0	192	18.0	49.3
31	13.4	24.5	85	11.7	16.2	139	12.9	21.6	193	12.5	20.0
32	13.1	19.8	86	14.0	29.4	140	13.9	27.3	194	22.0	99.7
33	16.9	43.0	87	15.2	37.0	141	14.8	32.1	195	13.2	25.5
34	15.9	35.9	88	16.0	31.2	142	15.5	26.6	196	12.5	18.0
35	13.4	21.8	89	16.0	40.2	143	16.2	43.3	197	14.5	26.5
36	17.5	59.7	90	14.5	25.5	144	13.5	23.4	198	19.0	60.0
37	16.4	41.1	91	15.0	30.0	145	10.7	11.1	199	17.5	47.0
38	19.0	50.3	92	20.5	66.1	146	17.8	54.0	200	15.0	24.0
39	16.0	38.5	93	15.0	27.2	147	21.7	87.5	201	16.0	35.8
40	17.0	28.4	94	17.5	53.9	148	15.0	30.2	202	16.5	39.3
41	13.5	24.9	95	17.9	52.5	149	13.2	23.3	203	19.8	77.6
42	11.5	17.3	96	17.5	58.7	150	19.5	74.1	204	15.8	36.0
43	16.5	38.9	97	16.7	36.1	151	19.0	63.0	205	13.0	24.3
44	15.4	31.9	98	15.5	32.0	152	12.3	16.0	206	12.7	19.4
45	18.8	64.2	99	12.3	20.6	153	12.7	18.9	207	14.4	24.2
46	12.0	21.9	100	15.9	37.9	154	16.0	34.0	208	16.9	45.1
47	14.6	31.2	101	16.0	35.4	155	16.2	38.5	209	19.0	61.2
48	20.0	67.8	102	13.5	23.5	156	12.8	19.1	210	18.2	50.7
49	13.8	26.3	103	21.5	96.3	157	16.3	39.8	211	12.1	16.9
50	14.7	34.6	104	14.0	22.8	158	14.7	27.3	212	12.5	20.9
51	17.3	56.9	105	12.0	15.0	159	11.5	13.6	213	10.2	11.4
52	15.5	35.4	106	19.0	62.3	160	16.5	42.6	214	13.5	20.7
53	21.5	94.3	107	11.5	16.2	161	12.0	14.4	215	15.1	30.6
54	14.5	28.7	108	22.0	86.9	162	14.6	30.4			

Appendix Table A-2. Data from the behavioral-attraction test in which male, female, and juvenile brook trout (*Salvelinus fontinalis*) were given a choice of well water (control) and well water in which mature male brook trout had been held. Side code indicates to which side of the exposure chamber the attractant was added: 1 = right, 2 = left. Fish with an asterisk (*) after their replicate number were excluded from the data analysis.

Rep. #	Attrac-tant	Gender	Length (mm)	Weight (g)	Test set #	# Trips during acclim. period	# Trips during test period	% of Time in Attrac-tant	% of Time in Control	Chamber	Side code
1	Fish	Male	145	28	1		7	61	39	3	1
2	Fish	Male	176	75	2		27	78	22	2	2
3	Fish	Male	129	29	3-1		25	48	52	3	1
4*	Fish	Male	156	53	3-2	0	2	13	87	2	1
5	Fish	Male	155	51	4-1		25	65	35	3	2
6	Fish	Male	182	89	4-2		10	46	54	1	2
7	Fish	Male	157	49	5		6	69	31	2	1
8	Fish	Male	163	52	6		16	76	24	3	2
9	Fish	Male	150	44	8		19	34	66	2	2
10	Fish	Male	157	57	9		20	68	32	3	1
1	Fish	Female	158	71	2		11	45	55	1	2
2	Fish	Female	146	49	5		19	44	56	1	1
3	Fish	Female	165	66	6		19	62	38	1	2
4	Fish	Female	155	57	8		14	85	15	3	2
5	Fish	Female	174	69	9		12	72	28	2	1
6	Fish	Female	152	51	10-1		15	42	59	3	2
7	Fish	Female	152	50	10-2		8	14	86	2	2
8	Fish	Female	166	66	11		11	21	79	2	1
9	Fish	Female	167	61	12		10	87	13	2	2
10	Fish	Female	138	43	13-1		25	64	36	3	1
11	Fish	Female	157	60	13-2		19	80	20	1	1
1	Fish	Juvenile	149	42	2		15	36	64	3	2
2*	Fish	Juvenile	134	36	3	0	2	99	1	1	1
3	Fish	Juvenile	144	46	4		5	60	40	2	2
4	Fish	Juvenile	133	35	5		15	45	55	3	1
5*	Fish	Juvenile	160	97	6	0	1	2	98	2	2
6	Fish	Juvenile	139	39	8		8	92	8	1	2
7	Fish	Juvenile	137	39	9		16	45	58	1	1
8*	Fish	Juvenile	129	25	10	0	3	96	4	1	2
9	Fish	Juvenile	137	35	11		24	71	29	1	1
10	Fish	Juvenile	131	32	12		15	82	18	3	2
11	Fish	Juvenile	121	27	13		13	86	14	2	1

Appendix Table A-3. Data from the behavioral-attraction test in which male, female, and juvenile brook trout (*Salvelinus fontinalis*) were given a choice of well water (control) and well water containing 10^{-10} M $PGF_{2\alpha}$. Side code indicates to which side of the exposure chamber the attractant was added: 1 = right, 2 = left. Fish with an asterisk (*) after their replicate number were excluded from the data analysis.

Rep. #	Attrac-tant	Gender	Length (mm)	Weight (g)	Test set #	# Trips during acclim. period	# Trips during test period	% of Time in Attrac-tant	% of Time in Control	Chamber	Side code
1	PGF2α	Male	146	44	1		5	25	75	3	2
2	PGF2α	Male	164	61	2		15	60	40	2	1
3	PGF2α	Male	183	71	3		12	25	75	2	2
4*	PGF2α	Male	133	29	4	0	2	33	67	3	1
5	PGF2α	Male	163	62	5		30	56	44	1	2
6	PGF2α	Male	123	27	6		21	64	36	2	1
7	PGF2α	Male	166	63	7		29	59	41	3	2
8*	PGF2α	Male	157	53	8	0	2	1	99	1	1
9	PGF2α	Male	147	46	9		11	17	83	3	2
10	PGF2α	Male	121	21	10		6	83	17	2	1
11	PGF2α	Male	168	66	12		13	80	20	3	1
12	PGF2α	Male	164	60	13		18	56	44	3	2
13	PGF2α	Male	112	20	14		12	51	49	1	1
14*	PGF2α	Male	162	54	16	0	2	74	26	3	1
15	PGF2α	Male	161	54	17		63	53	47	1	2
16	PGF2α	Male	144	38	19		16	42	58	1	2
1	PGF2α	Female	140	34	1		6	12	88	1	2
2	PGF2α	Female	146	36	2		4	11	89	1	1
3	PGF2α	Female	140	38	3		15	64	36	3	2
4	PGF2α	Female	121	56	4		18	62	38	1	1
5	PGF2α	Female	162	64	5		25	41	59	3	2
6	PGF2α	Female	154	45	6		23	45	55	1	1
7	PGF2α	Female	152	50	7		22	46	54	1	2
8*	PGF2α	Female	148	47	8	0	3	79	21	2	1
9	PGF2α	Female	164	59	9		34	46	54	1	2
10	PGF2α	Female	162	61	10		15	19	81	1	1
11	PGF2α	Female	147	45	12		11	18	82	2	1
12	PGF2α	Female	149	31	13		16	45	56	1	2
13	PGF2α	Female	154	52	15		10	54	46	3	2
14	PGF2α	Female	160	58	17		14	43	57	3	2
15	PGF2α	Female	156	50	18-1		15	18	82	3	1
16	PGF2α	Female	152	46	18-2		27	36	64	2	1
17*	PGF2α	Female	148	42	19	0	2	5	95	3	2
18	PGF2α	Female	136	35	20		14	32	68	3	1
1	PGF2α	Juvenile	161	50	1		16	72	28	2	2
2	PGF2α	Juvenile	132	26	3		19	38	62	1	2
3*	PGF2α	Juvenile	148	50	4	0	2	5	95	2	1
4	PGF2α	Juvenile	137	39	5		10	36	64	2	2
5	PGF2α	Juvenile	133	35	6		15	43	57	3	1
6	PGF2α	Juvenile	172	89	7		12	61	39	2	2
7	PGF2α	Juvenile	157	47	8		12	70	30	3	1
8*	PGF2α	Juvenile	133	34	9	0	3	6	94	2	2
9	PGF2α	Juvenile	112	20	11-1		13	69	31	3	2
10	PGF2α	Juvenile	151	35	11-2		8	15	85	1	2
11	PGF2α	Juvenile	140	39	12		41	53	47	1	1
12	PGF2α	Juvenile	133	36	13		12	22	78	2	2
13	PGF2α	Juvenile	137	32	14		13	71	29	2	1
14	PGF2α	Juvenile	121	24	17		22	24	76	2	2
15	PGF2α	Juvenile	173	83	19		10	23	77	2	2
16	PGF2α	Juvenile	140	35	20		16	25	75	2	1